Phaeobacterium nitratireducens gen. nov., sp. nov., a phototrophic gammaproteobacterium isolated from a mangrove forest sediment sample

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A novel brown-coloured, Gram-negative-staining, rod-shaped, motile, phototrophic, purple sulfur bacterium, designated strain AK40^T, was isolated in pure culture from a sediment sample collected from Coringa mangrove forest, India. Strain AK40^T contained bacteriochlorophyll *a* and carotenoids of the rhodopin series as major photosynthetic pigments. Strain AK40^T was able to grow photoheterotrophically and could utilize a number of organic substrates. It was unable to grow photoautotrophically and did not utilize sulfide or thiosulfate as electron donors. Thiamine and riboflavin were required for growth. The dominant fatty acids were $C_{12:0}$, $C_{16:0}$, $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The polar lipid profile of strain AK40^T was found to contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and eight unidentified lipids. Q-10 was the predominant respiratory quinone. The DNA G+C content of strain AK40^T was 65.5 mol%. 16S rRNA gene sequence comparisons indicated that the isolate represented a member of the family *Chromatiaceae* within the class *Gammaproteobacteria*. 16S rRNA gene sequence analysis indicated that strain AK40^T was closely related to *Phaeochromatium fluminis*, with 95.2 % pairwise sequence similarity to the type strain; sequence similarity to strains of other species of the family was 90.8–94.8 %. Based on the sequence comparison data, strain AK40^T was positioned distinctly outside the group formed by the genera *Phaeochromatium*, *Marichromatium*, *Halochromatium*, *Thiohalocapsa*, *Rhabdochromatium* and *Thiorhodovibrio*. Distinct morphological, physiological and genotypic differences from previously described taxa supported the classification of this isolate as a representative of a novel species in a new genus, for which the name *Phaeobacterium nitratireducens* gen. nov., sp. nov. is proposed. The type strain of *Phaeobacterium nitratireducens* is AK40^T (=JCM 19219^T=MTCC 11824^T).

Mangrove habitats are vital to humankind by virtue of their uses and aesthetic value. They serve as nurseries and feeding and spawning grounds for commercial fin fish and shellfish. Mangrove habitats are found in locations where a river enters the sea, and these dynamic ecosystems support numerous diversified soil microbes. Mangroves are found in many states of India, including Andhra Pradesh, the Andaman and Nicobar Islands, Goa, Gujarat, Karnataka, Kerala, Maharashtra, Tamil Nadu and West Bengal. The significance of microbes in aquatic ecosystems is well recognized as a result of many pioneer studies (Longhurst & Harrison, 1989; Wheeler & Kirchman, 1986). Microbes play different roles, including primary producers, secondary producers, consumers and decomposers. The activities of these microbes (photosynthetic, lithotrophic and organotrophic) are important for maintaining balance in the environment which, in turn, is vital for the maintenance of other life forms. A number of novel bacterial taxa have been identified from mangrove habitats.

All authors contributed equally to this study.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AK40^T is HF968497.

Five supplementary figures are available in the online Supplementary Material.

The family *Chromatiaceae* was proposed by Bavendamm (1924), with the type genus *Chromatium*, and its description was later emended by Imhoff (1984). Members of the *Chromatiaceae* are Gram-negative, ovoid, rod, spherical, spiral or vibrioid-shaped, motile by flagella or non-motile, multiply by binary fission, may contain gas vesicles and have internal photosynthetic membranes that are of either a tubular or vesicular type. Cells in the majority of the taxa contain photosynthetic pigments such as bacteriochlorophyll (BChl) *a* or *b* and carotenoids of the okenone, rhodopinal or spirilloxanthin groups (Takaichi, 1999). Cultures appear in different colours, including orange–brown to brownish red or pink, purple–red and purple–violet, depending on the type of carotenoids present. Cells grow under anoxic conditions in the presence of light (photolithoautotropically) with sulfide or sulfur as electron donor and deposit sulfur granules within the cells as an intermediate oxidation product that they may convert to sulfate; and can use hydrogen or reduced iron as an electron donor and also grow mixotrophically and photoassimilate a number of organic substrates. Some taxa are also capable of photo-organoheterotrophic growth. Most members of the *Chromatiaceae* are strictly anaerobic and obligately phototrophic, but others grow chemolithoautotrophically or chemo-organoheterotrophically under micro-oxic to oxic conditions in the dark. Carbon fixation is by the reductive pentose phosphate cycle in all species tested so far. Several taxa can fix N_2 and store elemental sulfur, poly-β-hydroxybutyrate, polysaccharides and polyphosphate. Some taxa may require different growth factors. Members of the *Chromatiaceae* commonly dwell in areas where anoxic conditions prevail with sulfide or sulfur sources with illumination, including diverse aquatic habitats and sediments such as ditches, estuaries, lakes, marine habitats, moist and muddy soils, ponds, rivers, salt and soda lakes and sulfur springs. The DNA G+C content varies between 45.5 and 70.4 mol% (Imhoff, 2005a).

In the course of bacterial diversity studies of mangrove samples, a bacterial strain, AK40^T, was isolated from a mangrove sediment sample collected near Coringa, Andhra Pradesh, India. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain AK40^T was closely related to the genus *Phaeochromatium* of the family *Chromatiaceae*. In this study, a polyphasic approach, including genotypic, phenotypic and chemotaxonomic characterizations, was used to determine the taxonomic position of strain AK40^T.

Strain AK40^T was isolated from a sediment sample. The temperature and pH of the sediment sample were 30 °C and 7.5, respectively. Anoxygenic phototrophic bacterium AK40^T was isolated using photolithoheterotrophic enrichments of the sediment sample, and subsequent purification was performed as described previously (Anil Kumar *et al.*, 2008a, b) in modified Pfennig medium (Pfennig & Trüper, 1992) supplemented with NaCl (2 %, w/v), pyruvate (0.3 %, w/v), sodium thiosulfate (2 mM) and ammonium chloride (0.12 %, w/v). Modified Pfennig medium was used throughout the study unless mentioned otherwise. Preservation was done at (80 °C in modified Pfennig broth with 20 % glycerol.

Colony morphology was studied after 72 h of anaerobic growth of the strain on modified Pfennig medium at 30 °C under 2000 lx illumination. The isolated pure colony was checked for cell morphology and motility by using phase-contrast microscopy (Olympus) at $\times 1000$ magnification and also by transmission electron microscope (JEOL JEM 2100) at an operating voltage of 200 kV. The Gram reaction was determined by using the Gram staining kit from HiMedia as described by the manufacturer.

Physiological and biochemical characteristics were determined as described previously (Anil Kumar *et al.*, 2008a, b; Srinivas *et al.*, 2006). The *in vivo* absorption spectrum was measured with a Spectronic Genesys 2 spectrophotometer in sucrose solution (Trüper & Pfennig, 1981). The absorption spectrum of pigments extracted with acetone was also recorded. Carotenoid composition was analysed by using C_{18}-HPLC (Takaichi & Shimada, 1992).

Strain AK40^T and *Phaeochromatium fluminis* JA418^T were grown anaerobically on modified Pfennig medium with 2 % NaCl at 30 °C under 2000 lx illumination for 3 days to prepare fatty acid methyl esters (classical methods) and were analysed using the Sherlock Microbial Identification System (MIDI-6890 with database TSBA50) by the protocol described by the manufacturer. At the time of harvesting, the cells were in the exponential phase of growth. Freeze-dried cells were analysed for polar lipids and quinones. Cells were extracted for polar lipid analysis (Bligh & Dyer, 1959) and analysed by two-dimensional TLC followed by spraying with appropriate detection reagents (5 % ethanolic molybdatophosphoric acid, molybdenum blue, ninhydrin and Molisch reagents) (Komagata & Suzuki, 1987). Genomic DNA was isolated by using the procedure of Marmur (1961) and the DNA G+C content was determined from melting-point (T_m) curves (Sly *et al.*, 1986) obtained by using a Lambda 35 spectrophotometer (Perkin Elmer) equipped with the Templab 2.0 software package.

For 16S rRNA gene sequencing, DNA was prepared using a bacterial DNA isolation kit (Qiagen). The 16S rRNA gene was amplified by PCR using universal bacterial primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-TACGGYTACCTTGTTACGACTT-3′). The PCR product was purified using a QIA quick PCR purification kit (Qiagen) and sequenced using an ABI PRISM model 3700 automatic DNA sequencer and Big Dye Terminator cycle sequencing kit (Applied Biosystems). The 16S rRNA gene sequence of strain AK40^T was subjected to BLAST sequence similarity search (Altschul *et al.*, 1990) and the EzTaxon-e server (Kim *et al.*, 2012) to identify the nearest taxa. Based on BLAST results, all 16S rRNA gene sequences of type strains of phototrophic members of genera belonging to the family *Chromatiaceae* were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov) and aligned

using the CLUSTAL W program in MEGA5 (Tamura *et al.*, 2011). Evolutionary history was inferred by using the maximum-likelihood (Tamura & Nei, 1993), neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Nei & Kumar, 2000) methods using the MEGA5 package (Tamura *et al.*, 2011). All positions containing gaps and missing data were eliminated. There was a total of 1355 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.*, 2011). A fast DNAML tree was reconstructed using the ARB software package (Ludwig *et al.*, 2004).

Cells of strain AK40^T were Gram-negative-staining, rod-shaped and motile and multiplied by binary fission. Cells were 1.0–4.0 μm long and 0.9–1.3 μm wide (Fig. 1). Colonies were 0.5–1.5 mm in diameter, circular, smooth, brown, opaque, convex and raised with entire margins on modified Pfennig medium. Strain AK40^T grew photo-organoheterotrophically (optimum light intensity 2000 lx). Photolithoautotrophic growth [anaerobic, light (2000 lx), $Na_2S/Na_2S_2O_3$ (0.5 mM), $NaHCO_3$ (0.1 %, w/v)], chemolithoautotrophic growth [aerobic, dark, $Na_2S_2O_3$ (0.5 mM), $NaHCO_3$ (0.1 %, w/v)], chemo-organoheterotrophic growth [aerobic, dark with glucose/pyruvate (0.3 %, w/v)] and fermentative growth [anaerobic, dark with glucose/pyruvate (0.3 %, w/v)] were not detected. NaCl was not required for growth, but was tolerated up to 7 % (w/v); the optimum NaCl concentration was 1.5–3 % (w/v). The pH range for growth of strain AK40^T was 5–9, with the optimum at pH 7.5. The temperature range was 22–37 °C and the optimum temperature for growth was 30 °C. Thiamine and riboflavin were required for growth. The colour of photosynthetically grown cell suspensions was brown. The whole-cell absorption spectrum (Fig. S1a) of strain AK40^T in sucrose solution gave maxima at 376, 488, 592, 702, 800 and 836 nm, confirming the presence of BChl *a* and carotenoids. The absorption spectrum of acetone-extracted pigments (Fig. S1b) gave maxima at 446, 476 and 506 nm, indicating the presence of carotenoids. The carotenoid composition of strain AK40^T, as determined by C_{18}-HPLC analysis, was lycopene (5 %), rhodopin (71 %), anhydrorhodovibrin (15 %), rhodovibrin (3 %) and spirilloxanthin (6 %) (Fig. S2). They were identified based on absorption spectra in the C_{18}-HPLC eluent of methanol and retention times on C_{18}-HPLC (Takaichi & Shimada, 1992). Other physiological characteristics are given in the species description and in Tables 1 and 2.

Fig. 1. Electron micrograph of negatively stained cells of strain AK40^T. Bar, 0.5 μm.

The cellular fatty acid composition of the strain AK40^T showed a profile of 11 fatty acids: $C_{12:0}$ (5.6 %), $C_{14:0}$ (0.6 %), $C_{16:0}$ (22.5 %), anteiso-$C_{16:0}$ (1.1 %), anteiso-$C_{17:0}$ (1.0 %), $C_{18:0}$ (0.7 %), $C_{18:1}$ 2-OH (1.9 %), $C_{18:1}\omega7c$ (32.0 %), $C_{18:3}\omega6,9,12c$ (2.8 %), summed feature 2 (one or more of $C_{12:0}$ aldehyde, iso-$C_{16:1}$ I and $C_{14:0}$ 3-OH; 0.9 %) and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2OH; 30.9 %). Unsaturated fatty acids constituted 65.7 % of the total. The major polar lipids consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and eight unidentified lipids (Fig. S3). Q-10 was the predominant respiratory quinone. The DNA G+C content of strain AK40^T was 65.5 mol% (T_m).

For phylogenetic assessment of strain AK40^T, 1464 bp of the 16S rRNA gene sequence was amplified and sequenced. The sequence was compared with the database of type strains of prokaryotic species with validly published names (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012), which revealed its closeness to *Phaeochromatium fluminis*. Based on pairwise sequence similarity, strain AK40^T was most closely related to *Phaeochromatium fluminis*, with 95.2 % similarity to the type strain, and to other members of the family *Chromatiaceae* (90.8–94.8 % sequence similarity). A phylogenetic tree reconstructed using the maximum-likelihood method showed the clustering of strain AK40^T with *Phaeochromatium fluminis* and a clade comprising *Marichromatium gracile*, *Halochromatium salexigens*, *Thiohalocapsa halophila*, *Rhabdochromatium marinum* and *Thiorhodovibrio winogradskyi* (Fig. 2). In the neighbour-joining phylogenetic tree and the ARB fast DNAML tree, strain AK40^T clustered with these six taxa (Figs S4 and S5).

Based on the BLAST result, strain AK40^T was compared with *Phaeochromatium fluminis* JA418^T, which is the nearest neighbour, and a number of differences were observed (Table 1). For instance, strain AK40^T differed from *Phaeochromatium fluminis* JA418^T with respect to cell size, the presence of sulfur granules, colour of cell suspension, NaCl range, temperature and pH growth range and optima, growth modes, vitamin requirement, sulfide tolerance, carbon substrate utilization, nitrogen and sulfur sources, major fatty acids, polar lipids and DNA G+C content (Table 1). Based on the phylogenetic analysis, a comparison was made between the characteristics of strain AK40^T and those of the type strains of all phototrophic genera of the family *Chromatiaceae* (Table 2), and a number of differences

Table 1. Phenotypic features that distinguish strain AK40^T from the closely related type strain *Phaeochromatium fluminis* JA418^T

Data for strain AK40^T and *Phaeochromatium fluminis* JA418^T are from the present study unless indicated. Both strains were rod-shaped, Gram-negative-staining and motile, grew optimally at 30 °C and 1.5–3 % (w/v) NaCl and utilized acetate, fumarate, propionate, pyruvate and succinate as carbon sources and ammonium chloride, glutamine as nitrogen sources; sulfate, sulfide and thiosulfate as sulfur sources. Both strains produced carotenoids of the spirilloxanthin group. Neither strain grew under aerobic conditions or utilized glucose, glycerol or citrate as carbon sources. +, Substrate utilized/present; (+), weakly positive; −, substrate not utilized/absent. Organic substrate utilization was tested during photoheterotrophic growth. For testing carbon sources, minimal medium supplemented with the respective carbon source (5 mM) was used. For nitrogen sources, NH_4Cl was replaced by different nitrogen sources with a final concentration of 0.12 % (w/v). For sulfur sources, $MgSO_4.7H_2O$ was replaced by the respective sulfur source (sodium thiosulfate, sodium sulfide) at 2 mM.

Characteristic	AK40^T	*Phaeochromatium fluminis* JA418^T
Cell size (μm)	2–4 × 0.9–1.3	4–6 × 0.4–0.5
Sulfur granules per cell (*n*)	0	1–3
NaCl range (%, w/v)	0–7	0.5–6.5
pH range	5–9	6.5–10
pH optimum	7.5	8–8.5
Temperature range (°C)	22–37	25–30
Growth modes*	PLH, POH	PLH, PLA, POH
Colour of cell suspension	Brown	Reddish brown
Vitamin requirement	Thiamine, riboflavin	None
Other growth factor requirement	None	Yeast extract
Sulfide tolerance (mM)	1	4
Carbon/electron donors		
Oxoglutarate	−	(+)
Methanol	−	(+)
Fructose	−	+
Valerate	+	−
Malate	(+)	−
Sucrose	−	(+)
Nitrate as a nitrogen source	+	−
Major fatty acids†	$C_{12:0}$, $C_{16:0}$, $C_{18:1}\omega7c$, SF3	$C_{16:0}$, $C_{16:1}\omega7c$/$C_{16:1}\omega6c$, $C_{18:1}\omega7c$
Polar lipids‡	DPG, PE, PG, L1–8	DPG, PE, PG, AL1–2§
DNA G+C content (mol%)	65.5	71.4§

*PLA, Photolithoautotrophic; PLH, photolithoheterotrophic; POH, photo-organoheterotrophic.
†SF3, Summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or iso-$C_{15:0}$ 2-OH).
‡DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL, unidentified aminolipid; L, unidentified lipid.
§Data taken from Shivali *et al.* (2012).

were observed. Thus, the cumulative differences that strain AK40^T exhibited from the above closely related taxa unambiguously supported the creation of a new genus *Phaeobacterium* gen. nov., and a new species, for which the name *Phaeobacterium nitratireducens* sp. nov. is proposed.

Description of *Phaeobacterium* gen. nov.

Phaeobacterium (Phae.o.bac.te′ri.um. Gr. adj. *phaeos* brown; L. neut. n. *bacterium* a stick, staff, rod; N.L. neut. n. *Phaeobacterium* the brown rod).

Cells are Gram-negative-staining, rod-shaped, oxidase-positive, catalase-negative, brown-pigmented, motile and anaerobic. Grow photo-organoheterotrophically. The major fatty acids are $C_{12:0}$, $C_{16:0}$, $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The predominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and eight unidentified lipids. Q-10 is the predominant respiratory quinone. The DNA G+C content of the type strain of the type species is 65.5 mol%. The genus is a member of the family *Chromatiaceae* of the class *Gammaproteobacteria* in the phylum *Proteobacteria*. The type species is *Phaeobacterium nitratireducens*.

Description of *Phaeobacterium nitratireducens* sp. nov.

Phaeobacterium nitratireducens (ni.tra′ti.re.du′cens. N.L. n. *nitras -atis* nitrate; L. part. adj. *reducens* leading back, bringing back and, in chemistry, converting to a reduced oxidation state; N.L. part. adj. *nitratireducens* reducing nitrate).

Exhibits the following properties in addition to those given in the genus description. Cells are 1.0–4.0 μm long and

Table 2. Phenotypic features that distinguish strain AK40^T from the type species of genera of the *Chromatiaceae*

Taxa: 1, strain AK40^T; 2, *Allochromatium* (data from Imhoff, 2005b); 3, *Halochromatium* (Imhoff & Caumette, 2005a); 4, *Isochromatium* (Imhoff, 2005c); 5, *Lamprocystis* (Imhoff, 2001); 6, *Marichromatium* (Imhoff, 2005d); 7, *Nitrosococcus* (Koops & Roser, 2005); 8, *Phaeochromatium* (Sucharita *et al.*, 2010; Shivali *et al.*, 2012); 9, *Rhabdochromatium* (Imhoff, 2005e), 10, *Rheinheimera* (Brettar *et al.*, 2002); 11, *Thermochromatium* (Imhoff & Madigan, 2005); 12, *Thioalkalicoccus* (Imhoff, 2005f); 13, *Thiobaca* (Rees *et al.*, 2002); 14, *Thiocapsa* (Imhoff & Caumette, 2005b); 15, *Thiococcus* (Imhoff, 2005g); 16, *Thiocystis* (Imhoff, 2005h); 17, *Thiodictyon* (Imhoff, 2005i); 18, *Thioflavicoccus* (Imhoff & Pfennig, 2001); 19, *Thiohalocapsa* (Imhoff & Caumette, 2005c); 20, *Thiolamprovum* (Guyoneaud *et al.*, 1998); 21, *Thiopedia* (Imhoff, 2005j); 22, *Thiophaeococcus* (Anil Kumar *et al.*, 2008b); 23, *Thiorhodococcus* (Guyoneaud *et al.*, 1997); 24, *Thiorhodovibrio* (Overmann *et al.*, 1992); 25, *Thiospirillum* (Imhoff, 2005k). +, Present; −, absent; ±, variable; NA, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Cell shape*	R	R	RO	R	SO	R	SE	R	R	RC	R	SO	R	S	S	R, SO	R	CO	S	SO	SO	S	SO	VS	RS
Cell length (μm) 2.5–4	2–4	1–4	2–4	3.5–4.5	2–3.5	1–1.7	1.5–2.5	0.4–0.5	1.5–1.7	0.5–1.5	1–2	1.3–1.8	1.6	1.2–3	1.2–1.5	2.5–3	1.5–2	0.8–1	1.5–2.5	2	2–2.5	2–2.5	1–2.0	1.2–1.6	
Motility	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+	−	+	−	−	−	+	+	+	+
Slime capsule	−	−	−	−	+	−	NA	NA	NA	NA	NA	−	NA	−	−	+	NA	−	+	−	−	−	−	NA	NA
Gas vesicles	−	−	−	−	+	−	NA	NA	NA	NA	−	−	−	±	−	−	+	−	−	+	+	−	−	NA	NA
Aggregate formation†	−	−	−	−	B	Spc	Si, P	−	−	−	−	−	Si, P	Tet	−	Irr	Irr	−	−	Plt	Rec	−	Si, P, Irr	−	−
Internal photosynthetic membranes‡	V	V	V	V	V	V	EI	V	V	−	V	T	V	V	T	V	V	T	V	V	V	V	V	V	V
Colour of cell suspension§	RB	OB	PR	PV–PUV	PV	BR, PUR	NA	DB	BOB	DBL–LBL	NA	YBr, OB	BR	PI, RR	OB	PUV	PUV	YBg–OB	PUR	PI, RR	PUR	S–B	BO	P	Y–OB
BChl	*a*	*a*	*a*	*a*	*a*	*a*	−	*a*	*a*	−	*a*	*b*	*a*	*a*	*b*	*a*	*a*	*b*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
Carotenoid group(s)‖	RN	RA	SP	RA	RA	SP, OK	NA	RN	LY	NA	SP	THS	LY	SP	THS	RA	RA	THS	OK	SP	OK	LY	SP	SP	LY, RN
pH optimum	7.5	6.5–7.6	7.2–7.6	6.5–7.6	7.0–7.3	6.5–7.6	NA	6.5–7.5	7.0	NA	7.0	8.8–9.2	NA	7.3	6.5 −7.5	6.5 −7.6	6.7–7.3	6.5–7.5	6.5–7.5	7.4–7.6	7.3–7.5	7.5	7.0–7.2	7.0–7.4	7.0
Temperature range (°C) 20–25	22–37	25–35	20–35	25–30	20–30	25–37	NA	NA	20–35	4–30	48–50	25	25–30	20–35	20–35	25–35	20–30	20–30	20–30	37	20	20–35	30–35	14–37	
Salt requirement	−	−	+	+	−	+	+	+	+	−	−	+	−	−¶	±	−	−	+	+	−	−	+	−	+	−
Growth mode(s)#	POH	PLA,	PLA, CLA, COH	PLA	PLA, CLA	PLA, CLA, COH	CLH	PLH	PLA	COH	PLA	PLA	PLH, POH	PLA, POH, CLA, COH	PLA	PLA, CLA, COH	PLA	PLA	PLA, POH, CLA, COH	PLA, CLA	PLA	PLA	PLA, CLA	PLA	PLA
Sulfate assimilation	−	+	+	+	NA	+	NA	NA	+	−	+	NA	−	+	−	±	NA	+	−	−	−	−	−	+	+
Vitamin B_{12} requirement	−	±	±	+	NA	−	NA	−	NA	NA	−	−	NA	−	−	−	NA	−	+	−	−	−	−	−	+
DNA G+C content (mol%)	65.5	55.1–66.3	64.6–66.3	62.2–62.8	63.4–64.1	68.9–70.4	50.5	68–69	60.4	47.8–48.9	60–63	63.6–64.8	63	63.3–66.3	69.4–69.9	61.3–67.9	65.3–66.3	66.5	65.9–66.6	64.5–66.5	62.5–63.5	68.5	66.8–67.0	61.0	45.5

*CO, Coccoid; R, rod; RC, rod to coccoid; RO, rod or ovoid to spherical; RS, rod to spiral; S, sphere; SE, spherical to ellipsoidal; SO, spherical to oval; VS, vibrioid to spirilloid.

†B, Branching; Irr, Irregular; Plt, platelet; Rec, rectangular; Spc, single, pairs or clumps; Si, single; P, pairs; Tet, tetrads.

‡EI, Extensive intracytoplasmic membrane systems arranged centrally in the protoplasm; T, tubular; V, vesicular.

§BO, Brown–orange; BOB, beige to orange–brown; BR, brownish; DB, dark brown; DBL, dark blue; LBL, light blue; OB, orange brownish; PI, pinkish; PR, pinkish red; PUR, purple–red; PUV, purple–violet; PV, pinkish violet; RB, reddish brown; RR, rose red; S–B, sandy brown–baker's chocolate; YBr, yellowish brown; YBg, yellow–beige.

‖LY, Lycopenal; OK, okenone; RA, rhodopinal; RN, rhodopin; SP, spirilloxanthin; THS, tetrahydrospirilloxanthin.

¶Marine strains may tolerate low concentrations of NaCl.

#CLA, Chemolithoautotrophic; CLH, chemolithoheterotrophic; COH, chemo-organoheterotrophic; PLA, photolithoautotrophic; PLH, photolithoheterotrophic; POH, photo-organoheterotrophic.

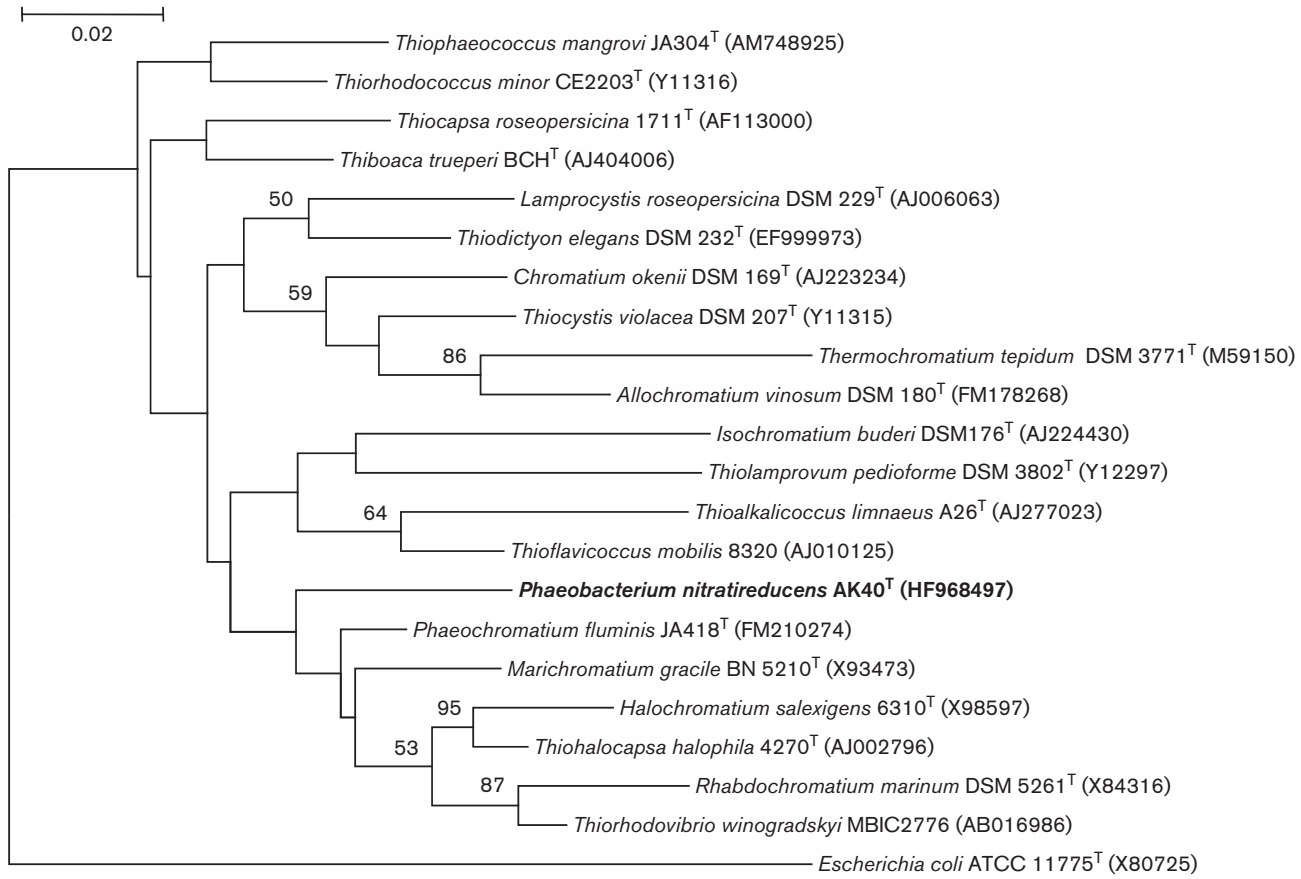

Fig. 2. Maximum-likelihood tree based on 16S rRNA gene sequences representing the phylogenetic relationships between strain AK40^T and the type species of phototrophic genera belonging to the family *Chromatiaceae*. Numbers at nodes are bootstrap values $\geqslant$50 %. *Escherichia coli* ATCC 11775^T was taken as the outgroup. Percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bar, 0.02 substitutions per nucleotide position.

0.9–1.3 μm wide, and they occur singly. Internal photosynthetic membranes are of the vesicular type. Colonies grown for 3 days at 30 °C on modified Pfennig medium are circular, 0.5–1.5 mm in diameter, smooth, brown, opaque and raised with entire margins. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 376, 488, 592, 702, 800 and 836 nm. The absorption spectrum of acetone-extracted pigments gives absorption maxima at 446, 476 and 506 nm. Major photosynthetic pigments are BChl *a* and rhodopin. The type strain is mesophilic (range 22–37 °C, optimum 30 °C), can grow under both acidic and alkaline conditions (pH 5.0–9.0, optimum pH 7.5) and does not require NaCl for growth, but can tolerate concentrations of NaCl up to 7 % (w/v), with optimum growth at 1.5–3 % NaCl. Growth occurs under anaerobic conditions in the light (photo-organoheterotrophy). Photoautotrophic and chemoautotrophic growth is not possible in the presence of sulfide and thiosulfate as electron donor and $NaHCO_3$ as carbon source. Chemo-organoheterotrophic growth is not possible in the presence of pyruvate as carbon source under aerobic conditions in the dark, and fermentative growth is not observed under anaerobic dark conditions in the presence of pyruvate. Photo-organoheterotrophy with various organic compounds is the preferred growth mode. Substrates that are utilized as carbon source/electron donors under photoheterotrophic conditions include acetate, fumarate, glycolate, malate, propionate, pyruvate, succinate, valerate and yeast extract but not butyrate, citrate, crotonate, fructose, glucose, glycerol, methanol, sucrose, Casamino acids or trehalose. Ammonium chloride, glutamine, nitrate and yeast extract are utilized as nitrogen sources, but not nitrite. Sulfate (2 mM), thiosulfate (2 mM) and sulfide (1.0 mM) are utilized as sulfur sources under photoheterotrophic conditions, but not sulfur. Ferrous iron cannot act as an electron donor. Thiamine and riboflavin are required for growth.

The type strain, AK40^T (=JCM 19219^T=MTCC 11824^T), was isolated from a sediment sample collected from Coringa mangrove forest, Andhra Pradesh, India.

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